Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 5997–6001, August 1988
Evolution

Western equine encephalitis virus is a recombinant virus

(RNA recombination/*Alphavirus*/evolution of RNA viruses)

CHANG S. HAHN, SHLOMO LUSTIG*, ELLEN G. STRAUSS, AND JAMES H. STRAUSS†

Division of Biology, 156-29, California Institute of Technology, Pasadena, CA 91125

Communicated by James Bonner, April 14, 1988

ABSTRACT **The alphaviruses are a group of 26 mosquito-borne viruses that cause a variety of human diseases. Many of the New World alphaviruses cause encephalitis, whereas the Old World viruses more typically cause fever, rash, and arthralgia. The genome is a single-stranded nonsegmented RNA molecule of + polarity; it is about 11,700 nucleotides in length. Several alphavirus genomes have been sequenced in whole or in part, and these sequences demonstrate that alphaviruses have descended from a common ancestor by divergent evolution. We have now obtained the sequence of the 3′-terminal 4288 nucleotides of the RNA of the New World *Alphavirus* western equine encephalitis virus (WEEV). Comparisons of the nucleotide and amino acid sequences of WEEV with those of other alphaviruses clearly show that WEEV is recombinant. The sequences of the capsid protein and of the (untranslated) 3′-terminal 80 nucleotides of WEEV are closely related to the corresponding sequences of the New World *Alphavirus* eastern equine encephalitis virus (EEEV), whereas the sequences of glycoproteins E2 and E1 of WEEV are more closely related to those of an Old World virus, Sindbis virus. Thus, WEEV appears to have arisen by recombination between an EEEV-like virus and a Sindbis-like virus to give rise to a new virus with the encephalogenic properties of EEEV but the antigenic specificity of Sindbis virus. There has been speculation that recombination might play an important role in the evolution of RNA viruses. The current finding that a widespread and successful RNA virus is recombinant provides support for such an hypothesis.**

The 26 members of the *Alphavirus* genus of the family Togaviridae are mosquito-borne viruses that form an important group of disease agents (1–3). The New World alphaviruses include western equine encephalitis virus (WEEV) and eastern equine encephalitis virus (EEEV), both of which are capable of causing encephalitis in humans and causing severe disease in horses. WEEV has a wide geographic distribution, being found from western Canada to Mexico and, discontinuously, to Argentina. WEEV is transmitted in the western United States by the mosquito *Culex tarsalis*; birds serve as an important vertebrate reservoir. In the eastern United States, WEEV is replaced by Highlands J virus (HJV), whose primary vector is *Culiseta melanura*. From serological studies (3, 4) and from limited sequencing studies (5, 6), WEEV and HJV are known to be very closely related, and HJV can be considered to be a strain of WEEV (2). In the eastern United States, the range of HJV overlaps that of EEEV, whose primary vector is also *Cs. melanura*. Other New World alphaviruses include Venezuelan equine encephalitis virus (VEEV), found in Central and South America; Fort Morgan virus, found in Colorado; and Aura virus, found in South America.

The Old World alphaviruses include Sindbis virus, the prototype alphavirus; Semliki Forest virus; Chikungunya virus; O'Nyong-nyong virus; and Ross River virus. Sindbis and Semliki Forest viruses have been intensively studied as models for alphavirus replication (7). Sindbis virus is widely distributed, being found in Europe, India, southeast Asia, Australia, and Africa. Close relatives of this virus, such as Ockelbo virus in Europe (8) and Babanki virus in Africa, cause disease in humans characterized by fever, rash, and arthritis. Chikungunya and O'Nyong-nyong viruses have caused large epidemics in Africa of a dengue-like disease also characterized by fever, rash, and arthralgia. Ross River virus is the causative agent of epidemic polyarthritis in Australia and the South Pacific.

Complete or partial RNA sequences have been obtained for Sindbis virus (9), Semliki Forest virus (10–12), Ross River virus (13), EEEV (14), and VEEV (15). Comparison of these nucleotide sequences and their encoded amino acid sequences has demonstrated that the alphaviruses are related by linear descent from a common ancestor (7). The relationships found are compatible, for the most part, with those derived from studies of serological cross-reactivity, which depends only upon antigenic epitopes in the structural proteins. In serological studies, however, WEEV has always been something of a puzzle. It is a New World virus that often causes encephalitis, but serologically it is most closely related to Sindbis virus, an Old World alphavirus not normally associated with encephalitis. To explore the relationship of WEEV to other alphaviruses, we have obtained the sequence of the 3′-terminal 4288 nucleotides of the WEEV genome‡ and found that WEEV appears to have arisen by recombination between an EEEV-like virus and a Sindbis-like virus.

MATERIALS AND METHODS

Virus RNA Preparation. WEEV RNA [strain BFS1703, isolated from *Cx. tarsalis* in July 1953 in Kern County, California (16)] was obtained from Mark Stanley and James Hardy (University of California, Berkeley). The virus had been passed twice by i.c. inoculation of suckling mice and four times (including three plaque isolations) in VERO cells. For RNA preparation, virus grown in VERO cells was purified by pelleting onto a 30% sucrose cushion followed by isopycnic banding in Nycodenz (Nyegaard, Oslo). After pelleting and dissociation in $NaDodSO_4$, the RNA was extracted by phenol/chloroform treatment, precipitated with ethanol, purified on a discontinuous sucrose gradient, and concentrated by ethanol precipitation.

Cloning and Sequencing. Clones containing the 3′-terminal 4288 nucleotides of WEEV RNA were obtained by using an oligo(dT)-tailed vector as a primer as described (17). Clones

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: WEEV, western encephalitis virus; EEEV, eastern encephalitis virus; VEEV, Venezuelan equine encephalitis virus; HJV, Highlands J virus.

*Present address: Israel Institute for Biological Research, P.O. Box 19, Ness-Ziona, 70450, Israel.
†To whom reprint requests should be addressed.

‡The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03854).

were sequenced by using the chemical sequencing method (18, 19).

RESULTS

Partial Sequence of WEEV RNA. The translated sequence of the 3′-terminal 4170 nucleotides of the WEEV genome is shown in Fig. 1. This sequence begins in the region encoding the carboxyl terminus of nonstructural protein 4, continues through the junction region between the nonstructural and structural proteins containing the start of the subgenomic mRNA that is translated to give the structural proteins (20), and progresses through the coding sequence of the three structural proteins of the virus (a nucleocapsid protein, C, and two envelope glycoproteins, E2 and E1) and finally through the 3′-terminal untranslated sequence, which ends in a poly(A) tract.

We have previously sequenced the amino termini of the three structural proteins of the McMillan strain of WEEV (isolated in 1941 in Canada from the brain of a fatal human case) and thus established the start points of the structural proteins (21). Comparison of the amino acid sequence of the McMillan strain with that deduced here for the BFS1703 strain (isolated from mosquitos in 1953 in California) reveals four amino acid differences in 142 amino acids for which comparison is possible (one in C, one in E2, and two in E1). However, reevaluation of the original data for the McMillan strain suggests that the apparent difference in the capsid proteins may result from a misscall in the McMillan sequence and that there are no differences between the capsid proteins

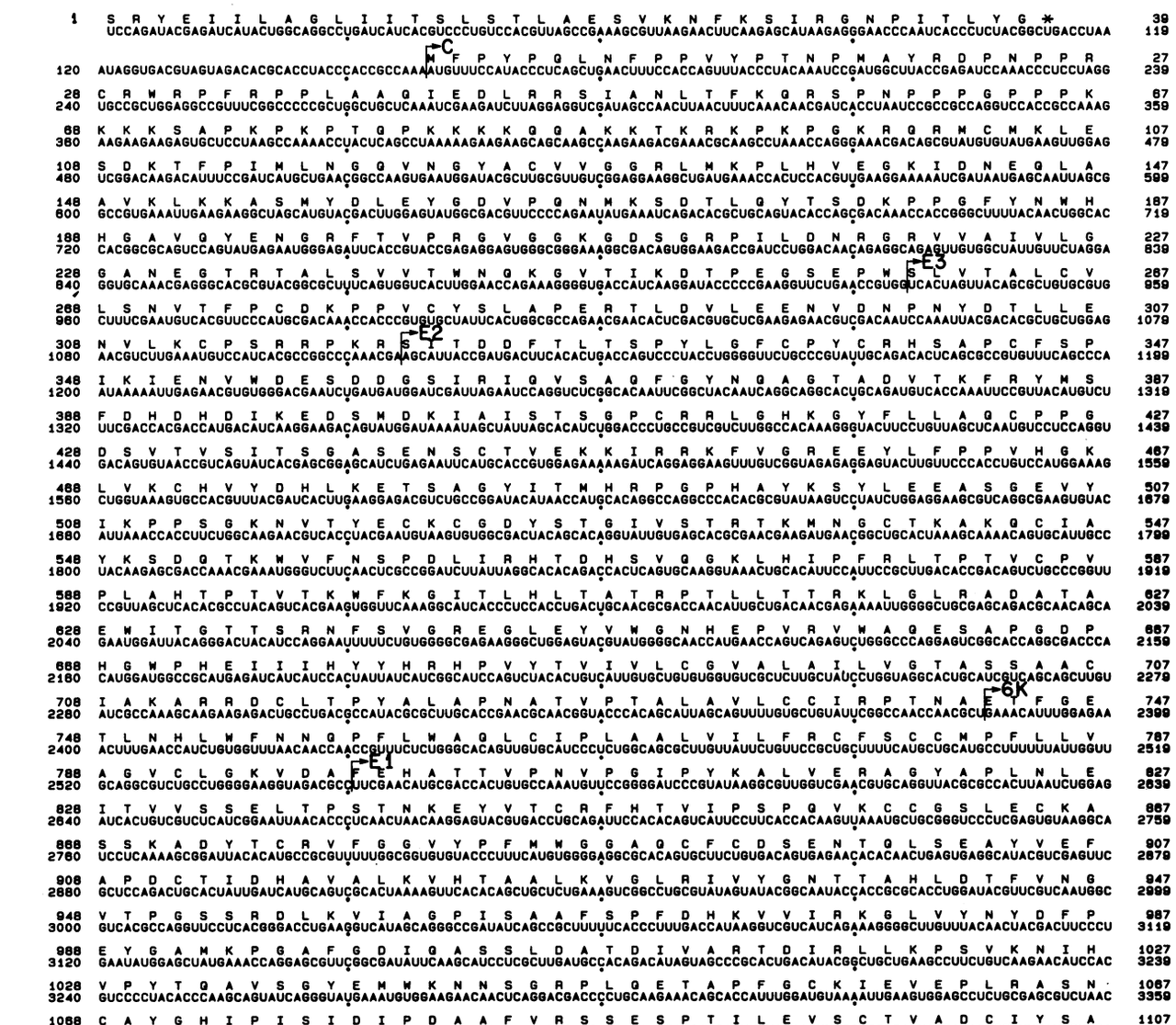

FIG. 1. Sequence of the 3′-terminal 4170 nucleotides of WEEV RNA (strain BFS1703). The start points of the structural proteins are indicated. Asterisks indicate the termination codons for the nonstructural and structural polyproteins. Two independent clones were sequenced and only one clonal difference was found: the GAC encoding Asp-72 of E2 was replaced by a UAC encoding tyrosine in the second clone.

in the residues compared. The amino acid sequence divergence between the two strains is 2.8% (or 2.1% when the apparent difference in the capsid proteins is discounted). We also have reported the sequence of the 3′-terminal 351 nucleotides of McMillan RNA (6). Comparison with that for BFS1703 shows three nucleotide substitutions and one deletion (in McMillan) between these two strains, a divergence of 1.1%. These comparisons establish the fact that the widely studied McMillan strain (the prototype WEEV virus) and the BFS1703 strain are the same virus. Since these two strains were isolated 12 years apart in different geographic areas, the rate of divergence of WEEV in nature is at most 0.1–0.2% per year, which is low in comparison to rates that have been established for several RNA viruses (22, 23).

WEEV Is a Recombinant. The amino acid sequences of the WEEV structural proteins are compared to those of EEEV and of Sindbis virus in Fig. 2. Inspection of this figure clearly reveals that the WEEV capsid protein C is most closely related to that of EEEV, whereas the glycoproteins E2 and E1 are more closely related to the corresponding proteins of Sindbis virus.

The relationships among the proteins of these viruses are summarized in Table 1. The amino-terminal and carboxyl-terminal domains of the capsid protein are considered separately because of the fact that the carboxyl termini of all alphavirus capsid proteins are closely related. The capsid proteins of WEEV and EEEV are much more closely related (85% sequence identity) than are those of WEEV and Sindbis virus (53% identity). The relationships are reversed in the case of the envelope proteins. The envelope proteins of WEEV and Sindbis virus are much more closely related (71% identity overall) than are those of WEEV and EEEV (46% identity). Figures for the carboxyl-terminal domain of nsP4 are also included. Although this protein is highly conserved among alphaviruses, its carboxyl-terminal domain is more variable, and WEEV and EEV are much more closely related in this region than are WEEV and Sindbis virus.

Also included in Table 1 are comparisons with another alphavirus, VEEV, to illustrate that alphaviruses in general

Table 1. Percent sequence identity among WEEV, EEEV, VEEV, and Sindbis virus (SINV) proteins

	WEEV EEEV	WEEV SINV	EEEV SINV	EEEV VEEV	SINV VEEV	WEEV VEEV
nsP4 (C terminus)	**70**	35	40			
Capsid						
N terminus*	**78**	39	36	42	27	49
C terminus*	**91**	69	64	76	61	77
Overall	**85**	53	50	59	44	63
Envelope						
E3	50	58	42	59	49	56
E2	44	**68**	42	46	40	41
6K	44	**67**	45	54	40	40
E1	49	**76**	51	58	51	50
Overall	47	**71**	46	53	46	46

*N terminus refers to amino acids 1–132 of the Sindbis capsid protein or the corresponding positions in the aligned files in Fig. 2. C terminus includes the remaining amino acids in the capsid proteins in the aligned files. Unusually high identity values are shown in boldface type.

differ from one another in a uniform and consistent way. Sequence data for Semliki Forest virus or Ross River virus lead to similar results (not shown). WEEV is exceptional in that it is closely related to Sindbis virus in the region of the genome encoding E1 and E2, but to EEEV in other regions.

Nucleotide sequences in the carboxyl-terminal region of nsP4 and in the junction region between structural and nonstructural proteins, which are believed to contain important regulatory elements for transcription of a subgenomic mRNA translated to produce the structural proteins (20), are compared for the three viruses in Fig. 3*a*. The EEEV and WEEV nucleotide sequences are very similar to one another and the sequences flanking the start of the subgenomic 26S RNA are identical. The sequence of Sindbis virus in this region is similar but not identical. The nsP4 proteins of EEEV

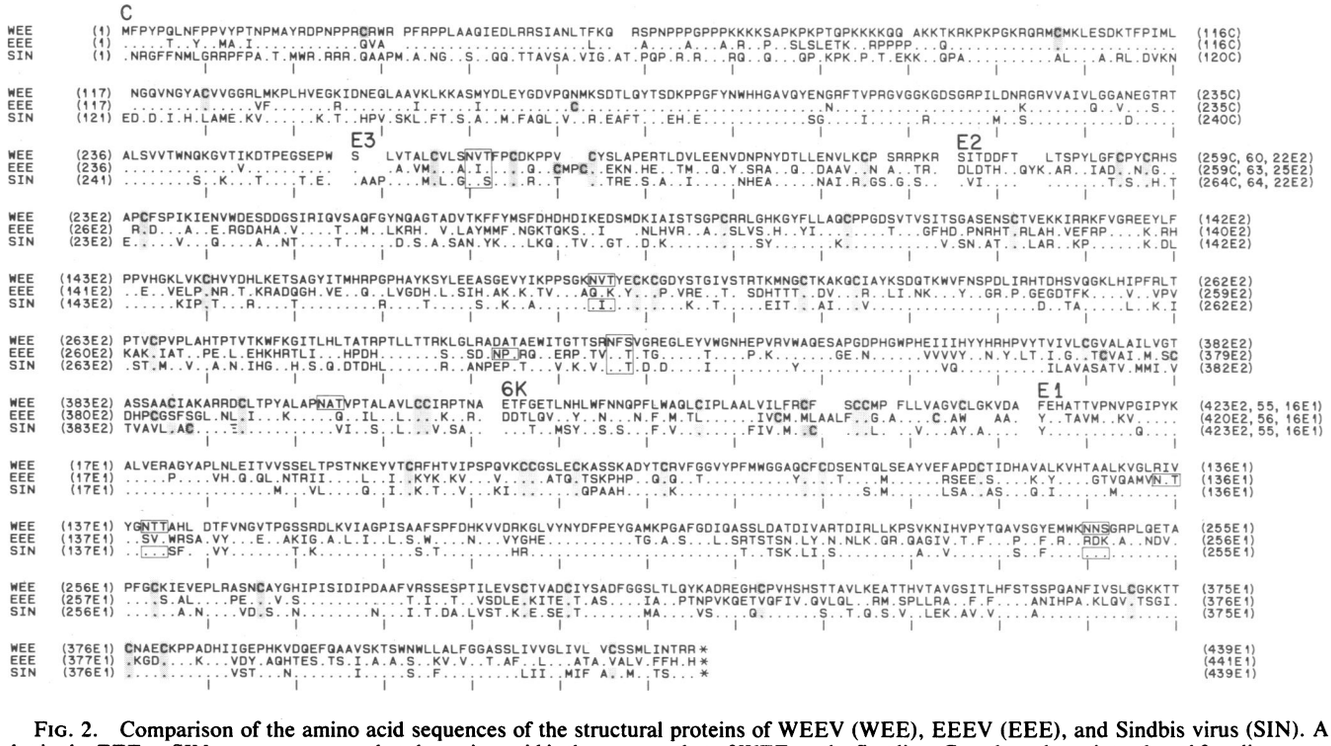

FIG. 2. Comparison of the amino acid sequences of the structural proteins of WEEV (WEE), EEEV (EEE), and Sindbis virus (SIN). A dot in the EEE or SIN sequence means that the amino acid is the same as that of WEE on the first line. Gaps have been introduced for alignment. Potential glycosylation sites are boxed and cysteines are highlighted with dotted overlay.

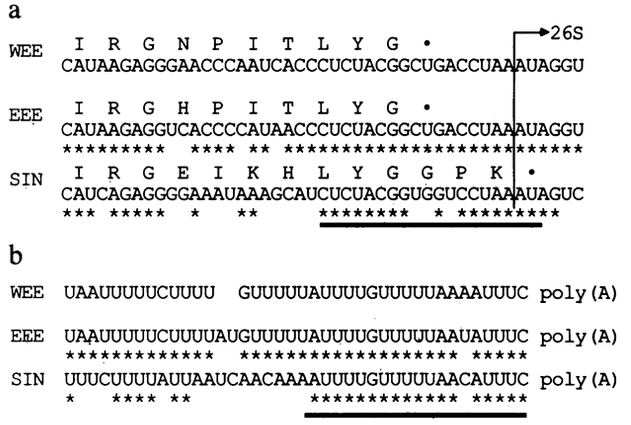

FIG. 3. Comparison of the nucleotide sequences in the junction regions of EEEV (EEE), WEEV (WEE), and Sindbis virus (SIN) (*a*) or at the 3′ end of the RNAs (*b*). Asterisks denote conserved nucleotides. The heavy underlines denote conserved nucleotide sequences in the alphaviruses that are believed to form important regulatory elements for RNA transcription (7). The termination codons that end the nonstructural open reading frames are marked with black circles.

and WEEV terminate at the same residue, whereas the Sindbis virus protein terminates downstream.

The sequences at the 3′ termini of WEEV, EEEV, and Sindbis virus are shown in Fig. 3*b*. The 3′-terminal 19 nucleotides have been proposed to form an important element in *Alphavirus* RNA replication because they are highly conserved among members of this genus (6), and this sequence element (underlined in Fig. 3*b*) is invariant among these three viruses with the exception of the sixth nucleotide from the end. The nucleotides upstream of this are A/U rich and not particularly conserved among alphaviruses, but in this domain the sequences of WEEV and EEEV are almost identical, whereas that of Sindbis virus is more variable.

These results show that within the region examined, the WEEV nucleotide sequence is recombinant, with both the 5′ and 3′ ends derived from an EEEV-like virus and the intervening glycoprotein genes derived from a Sindbis-like virus. We presume that the 5′-terminal two-thirds of the genome, which has not yet been sequenced, is also derived from the EEEV-like virus. Partial support for this comes from our previous finding that the 5′ terminal sequence of HJV is similar to that of EEEV (5).

The Recombination Events. Our interpretation of the sequence information is shown schematically in Fig. 4, which is included in part to illustrate the structure of the alphavirus genome. In this model, close inspection of the aligned sequences in Fig. 2 suggests that the 5′ crossover occurred in E3. Gaps must be introduced into the amino acid sequences to align them, and the two gaps of three amino acids each in E3 are of particular interest. The first gap, following residue 1, is shared by WEEV and EEEV; upstream of this WEEV and EEEV are in almost perfect alignment (only one gap of one amino acid must be introduced into each sequence to maintain alignment), whereas several gaps must be introduced to keep the Sindbis virus sequence aligned. Conversely, the gap following residue 21 of WEEV E3 is shared by Sindbis virus and WEEV; downstream of this, the Sindbis virus and WEEV sequences are in almost perfect register (only one gap of one amino acid is required to maintain alignment), whereas numerous gaps are required to keep the EEEV sequence in register. This suggests that the recombination event occurred between these two gaps in E3, which is compatible with the sequence similarities exhibited by the capsid proteins and the glycoproteins in Table 1.

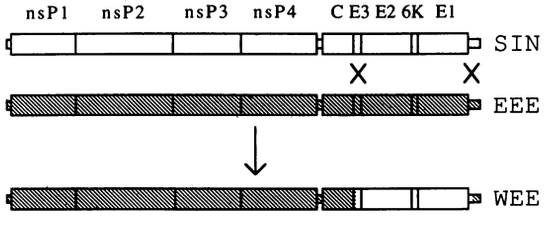

FIG. 4. Schematic representation of the recombination event that produced WEEV (WEE). The crossover points to produce WEE are indicated. SIN, Sindbis virus; EEE, EEEV.

The 3′ crossover appears to have occurred in the 3′ untranslated region. The 60 nucleotides of WEEV RNA following the structural protein stop codon are very similar to the Sindbis virus sequence, whereas the last 80 nucleotides of the RNA are similar to EEEV, with no sequence similarity detectable in between. Although a double crossover seems inherently less likely than a single crossover, the presence of important replication signals at the 3′ end may require such an event to produce viable (or at least efficiently replicating) virus (6, 7).

There is a formal possibility that WEEV is one of the parental viruses in a cross that resulted in the reciprocal recombinants Sindbis virus and EEEV. Because RNA recombination is believed to occur by a copy-choice mechanism, however, in which reciprocal recombinants are not produced (24), and because of the apparent rarity of viable recombinant viruses, this possibility appears remote.

Interaction of the Nucleocapsid and Glycoproteins During Virus Budding. Alphaviruses mature when preassembled nucleocapsids, which are icosahedral structures consisting of 180 copies of the nucleocapsid protein and one molecule of the virus RNA, acquire an envelope by budding through the plasma membrane (25, 26). The envelope consists of a lipid bilayer derived from the host cell in which are embedded two virus-encoded glycoproteins, E2 and E1. The nucleocapsid and the glycoproteins are thought to interact specifically with one another, so as to exclude nonvirus proteins from the structure; the free energy for driving virus budding is derived from these specific interactions. During evolution, certain domains of the glycoproteins of a particular virus must have been selected for maximal specific interaction with the capsid of that virus. In a recombinant virus that contains the capsid protein from one virus and the glycoproteins from another, the interactions during budding might not be optimal. During passage of such a recombinant virus, selection pressure would favor variants in which the nucleocapsid and glycoprotein interactions were improved. It is thus of considerable interest that there are only seven amino acid differences between WEEV and EEEV in the carboxyl-terminal 104 amino acids of the capsid protein, and for 6 of these WEEV has the Sindbis virus amino acid (Fig. 2). This suggests that this domain of the capsid protein interacts with the glycoproteins during virus assembly and that, after the recombination event, selection has led to some of the EEEV capsid amino acids being replaced with Sindbis virus amino acids to allow more efficient interaction with the Sindbis virus glycoproteins. Conversely, in the carboxyl-terminal 16 amino acids of E2, there are 6 amino acid differences between WEEV and Sindbis virus, and for 4 of these WEEV has the EEEV amino acid, suggesting by the same logic that this domain of E2 interacts with the capsid during budding. Other examples can be found in other regions of the structural proteins. The hypothesis that these domains are involved in capsid–glycoprotein interactions can be tested by site-specific mutagenesis (27).

DISCUSSION

The Origin of WEEV. The two parents of WEEV and the time of the recombination event cannot be determined at the current time. As described earlier, the McMillan strain of

WEEV isolated in 1941 in Canada and the BFS1703 strain isolated in 1953 in California are clearly strains of the same virus. They have nearly identical capsid proteins, glycoproteins E2 and E1, and 3'-terminal sequences. Thus, the recombination event could not have occurred during passage of the virus in culture, as this would have required the identical recombination event to have occurred twice, in different laboratories. By the same logic, the recombination event must have predated the isolation of the McMillan strain of WEEV in 1941. Furthermore, all of the sequence information obtained is compatible with the hypothesis that the recombinant virus arose before the separation of WEEV and HJV. On the other hand, the amino-terminal portions of the capsid proteins of WEEV and EEEV are very similar, a lysine- and arginine-rich domain not well conserved among alphaviruses (28). Thus, the similarity in the WEEV and EEEV sequences, together with the fact that RNA viruses diverge rapidly (21), suggests that the recombination event must be relatively recent. We propose that one of the parents was EEEV itself. The sequence similarities with Sindbis virus in the envelope protein regions are not as pronounced and suggest that the second parent was not Sindbis virus itself but a relative of it. Because WEEV and EEEV are New World viruses, we propose that the recombination event occurred in the New World between EEEV or an immediate ancestor of it and a Sindbis-like virus that has yet to be identified. It seems most likely that the recombination event took place in the mosquito vector, in which the virus sets up a persistent life-long infection. EEEV and HJV overlap in geographic ranges and mosquito vector. Thus HJV might represent the ancestral recombinant virus that radiated to produce WEEV.

Recombination in RNA Virus Evolution. There has been much speculation about the importance of recombination in the evolution of RNA viruses (29, 30). In segmented RNA viruses, reassortment of individual genome segments during mixed infection, a form of recombination equivalent to the shuffling of chromosomes in diploid creatures, is readily demonstrated in cell culture. Reassortment is a major mechanism for generating new pandemic strains of influenza virus (31, 32), and it may be that the ability to undergo ready recombination conveys significant selective advantage. Among the nonsegmented RNA viruses, recombination has been in general more difficult to demonstrate, but it has been shown to occur in the picornaviruses (33, 34), the coronaviruses (35, 36), and the bromoviruses (37), although not before now in the alphaviruses. In poliovirus, recombination occurs by a copy-choice mechanism during RNA replication (24), and it is assumed that all RNA recombination (as opposed to reassortment) occurs by this mechanism. Although well established in principle, evidence for the importance of recombination in nature as a mechanism that leads to successful new strains is limited. In the case of poliovirus, recombination has been shown to occur in vaccinees that have simultaneously received high doses of three attenuated viruses (34), but this is not a natural system. The finding that WEEV, a virus with a wide geographic range, is a naturally occurring recombinant lends support to the hypothesis that RNA recombination is an important force in the evolution of RNA viruses. In this particular case, it has given rise to a new virus that combines the disease-causing potential of EEEV with new antigenic properties from a Sindbis virus-like virus.

We thank Drs. M. Stanley and J. Hardy for the WEEV RNA used in this project. This work was supported by Grants AI20612 and AI10793 from the National Institutes of Health and Grant DMB86-17372 from the National Science Foundation.

1. Griffin, D. E. (1986) in *The Togaviridae and Flaviviridae*, eds. Schlesinger, S. & Schlesinger, M. (Plenum, New York), pp. 209–249.
2. Chamberlain, R. W. (1980) in *The Togaviruses*, ed. Schlesinger, R. W. (Academic, New York), pp. 175–227.
3. Calisher, C. H., Shope, R. E., Brandt, W., Casals, J., Karabatsos, N., Murphey, F. A., Tesh, R. B. & Wiebe, M. E. (1980) *Intervirology* **14**, 229–232.
4. Hayes, C. G. & Wallis, R. C. (1977) *Adv. Virus Res.* **21**, 37–83.
5. Ou, J.-H., Strauss, E. G. & Strauss, J. H. (1983) *J. Mol. Biol.* **168**, 1–15.
6. Ou, J.-H., Trent, D. W. & Strauss, J. H. (1982) *J. Mol. Biol.* **156**, 719–730.
7. Strauss, E. G. & Strauss, J. H. (1986) in *The Togaviridae and Flaviviridae*, eds. Schlesinger, S. & Schlesinger, M. (Plenum, New York), pp. 35–90.
8. Niklasson, B., Espmark, A., LeDuc, J. W., Gargan, T. P., Ennis, W. A., Tesh, R. B. & Main, A. J. (1984) *Am. J. Trop. Med. Hyg.* **33**, 1212–1217.
9. Strauss, E. G., Rice, C. M. & Strauss, J. H. (1984) *Virology* **133**, 92–110.
10. Garoff, H., Frischauf, A.-M., Simons, K., Lehrach, H. & Delius, H. (1980) *Nature (London)* **288**, 236–241.
11. Garoff, H., Frischauf, A.-M., Simons, K., Lehrach, H. & Delius, H. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6376–6380.
12. Takkinen, K. (1986) *Nucleic Acids Res.* **14**, 5667–5682.
13. Dalgarno, L., Rice, C. M. & Strauss, J. H. (1983) *Virology* **129**, 170–187.
14. Chang, G.-J. J. & Trent, D. W. (1987) *J. Gen. Virol.* **68**, 2129–2142.
15. Kinney, R. M., Johnson, R. J. B., Brown, V. C. & Trent, D. W. (1986) *Virology* **152**, 400–413.
16. Hardy, J. L., Reeves, W. C., Rush, W. A. & Nir, Y. D. (1974) *Infect. Immun.* **10**, 553–564.
17. Lindqvist, B. H., DiSalvo, J., Rice, C. M., Strauss, J. H. & Strauss, E. G. (1986) *Virology* **151**, 10–20.
18. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
19. Smith, D. R. & Calvo, J. M. (1980) *Nucleic Acids Res.* **8**, 2225–2274.
20. Ou, J.-H., Rice, C. M., Dalgarno, L., Strauss, E. G. & Strauss, J. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5235–5239.
21. Bell, J. R., Bond, M. W., Hunkapiller, M. W., Strauss, E. G., Strauss, J. H., Yamamoto, K. & Simizu, B. (1983) *J. Virology* **45**, 708–714.
22. Steinhauer, D. A. & Holland, J. J. (1987) *Annu. Rev. Microbiol.* **41**, 409–433.
23. Smith, D. B. & Inglis, S. C. (1987) *J. Gen. Virol.* **68**, 2729–2740.
24. Kirkegaard, K. & Baltimore, D. (1986) *Cell* **47**, 433–443.
25. Strauss, E. G. & Strauss, J. H. (1985) in *Virus Structure and Assembly*, ed. Casjens, S. (Jones & Bartlett, Boston), pp. 205–234.
26. Fuller, S. D. (1987) *Cell* **48**, 923–934.
27. Rice, C. M., Levis, R., Strauss, J. H. & Huang, H. V. (1987) *J. Virol.* **61**, 3809–3819.
28. Rice, C. M. & Strauss, J. H. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2062–2066.
29. Strauss, J. H. & Strauss, E. G. (1988) *Annu. Rev. Microbiol.* **42**, 657–683.
30. Hodgman, T. C. & Zimmern, D. (1988) in *RNA Genetics*, eds. Domingo, E., Holland, J. J. & Ahlquist, P. (CRC Press, Boca Raton, FL), Vol. 3, in press.
31. Desselberger, U., Nakajima, K., Alfino, P., Pederson, F. S., Haseltine, W. A., Hannoun, C. & Palese, P. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3341–3345.
32. Webster, R. G., Laver, W. G., Air, G. M. & Schild, G. C. (1982) *Nature (London)* **296**, 115–121.
33. Cooper, P. D. (1977) in *Comprehensive Virology*, eds. Fraenkel-Conrat, H. & Wagner, R. R. (Plenum, New York), Vol. 9, pp. 133–207.
34. Kew, O. M. & Nottay, B. K. (1984) in *Modern Approaches to Vaccines: Molecular and Chemical Basis of Virus Virulence*, ed. Channock, R. M. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 357–362.
35. Lai, M. M. C., Baric, R. S., Makino, S., Keck, J. G., Egbert, J., Leibowitz, J. L. & Stohlman, S. A. (1985) *J. Virol.* **56**, 449–456.
36. Makino, S., Keck, J. G., Stohlman, S. A. & Lai, M. M. C. (1986) *J. Virol.* **57**, 729–737.
37. Bujarski, J. J. & Kaesberg, P. (1986) *Nature (London)* **321**, 528–531.